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A Rapid Method for Isolating Glandular Trichomes

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ABSTRACT

A physical method is described for the rapid isolation of plant trichomes, with emphasis on stalked glandular types. The technique involved breaking frozen trichomes with powdered dry ice and collection of glandular heads by sieving from larger tissue fragments. This method was applied to several plants that bear similar stalked trichomes: geranium (*Pelargonium*), potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*), squash (*Cucurbita pepo*), and velvetleaf (*Abutilon theophrasti*). The tissue preparation was of sufficient quality without further purification for biochemical and molecular studies. The preparation maintained the biochemical integrity of the trichomes for active enzymes and usable nucleic acids. A large quantity of tissue can be harvested; for example, 351 milligrams dry weight of glandular trichomes were harvested from geranium pedicels in 12 hours. The utility of the technique was demonstrated by examining the fatty acid composition of tall glandular trichomes of geraniums, *Pelargonium* ×*hortorum* L.H. Bailey. These purified cells contained high concentrations of unusual ω5-unsaturated fatty acids, proportionally 23.4% of total fatty acids in the trichomes. When the trichomes were removed, the supporting tissue contained no ω5-fatty acids, thereby unequivocally localizing ω5-fatty acids to the trichomes. Because ω5-fatty acids are unique precursors for the biosynthesis of ω5-anacardic acids, we conclude that anacardic acid synthesis must occur in the glandular trichomes.

Glandular trichomes are modified epidermal hairs that produce chemical exudates. The exudates consist of a diverse group of allelochemicals associated with pest resistance in plants (reviews in refs. 16, 26, and 29). The exudate chemicals are potent enough to deter a variety of otherwise persistent herbivores (5, 13, 17). The glandular cell at the tip of the trichome, the head cell, displays many ultrastructural features indicative of active metabolism and secretion, leading to the general assumption that it is the site of synthesis (3, 6, 24); in tobacco and spearmint, the trichome's exudate synthesis was confirmed by biochemical evidence (12, 15).

Attempts to study the unique biochemistry of the head cell have been hampered by the difficulty of harvesting adequate quantities of tissue (29). Glandular trichomes constitute a diffuse tissue type, dispersed as cells on the distal ends of stalks, and are often interspersed with other plant pubescences. Various methods have been used to gather glandular trichome tissue for analysis: (a) plant surface shaved manually with a scalpel blade; (b) epidermis peeled to remove trichome-containing fragments (2, 21); (c) coverslip wiped over projecting trichomes to collect adhering cells (15); (d) cotton swab saturated with antioxidant wiped over leaflet surface to collect trichomes (20); (e) leaves submerged in buffer brushed gently to detach epidermal fragments with trichomes (4); (f) tissue fragmented in a blender and cell types separated by Percoll density gradient centrifugation (25); (g) leaves tumbled in a flask with small glass beads in buffer to mechanically detach epidermal fragments containing trichomes (11); and (h) leaf surface extensions sheared with small glass beads propelled in buffer by the rotor of a commercial cell disrupter (11).

The above methods were not suitable for our studies of glandular head protein and mRNA because we needed large quantities of pure trichome head cells without any possibility of biochemical degradation or contamination. We therefore developed an efficient method to isolate glandular trichome head cells that uses low temperatures to keep the cells frozen. We demonstrate the effectiveness of the method with photographs and the utility of the method with localization of a unique fatty acid.

The fatty acid, unusual because of its unsaturation at the ω5 position, was measured in trichomes because it is implicated in the biological activity of the geranium, *Pelargonium* ×*hortorum* L.H. Bailey. In geraniums, resistance to arthropod pests is mediated by the presence of a sticky exudate on the surface of glandular trichomes (31). This exudate contains anacardic acids, which in the resistant genotype consists mostly of ω5-unsaturated anacardic acids (10, 14). Anacardic acids are biosynthesized from fatty acid precursors, and the specific ω5 anacardic acid requires the presence of an ω5 fatty acid precursor for biosynthesis (30). We investigated the possibility that the trichome head cell was the site of this biosynthesis by localizing ω5 fatty acid precursor pools using the trichome isolation method.

MATERIALS AND METHODS

Plant Material

Various species of plants with glandular trichomes were harvested from field or greenhouse sources. The method was developed for garden geraniums, *Pelargonium* ×*hortorum* L.H. Bailey (an interspecific hybrid of unknown parentage, generally considered to be a species), but we also used it to obtain glandular trichomes from other plant material; specifically, tissues of potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*), squash (*Cucurbita pepo*), and the allelopathic weed, velvetleaf (*Abutilon theophrasti*). Because this

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technique worked successfully on all glandular trichome-containing plant material tested, it has broad potential, but for clarity we report the method only in terms of use with geranium.

Geraniums were grown in growth chambers under standard conditions. A microscopic examination of various plant parts revealed that the pedicel of the inflorescence contained a high density of tall glandular trichomes as the only plant pubescence. Tall glandular trichomes were also present on the leaf, petiole, sepal, and stem, but on these plant tissues, tall glandular trichomes were interspersed with two other plant pubescences: spines and short glandular trichomes. The preparation worked well on pedicels, leaves, petioles, sepals, and stems, but to investigate the secretory cell of the tall glandular trichome, we needed a pure preparation of head cells alone; therefore, in this case, we harvested and processed only pedicels.

Tissue Preparation

Fully and partially opened inflorescences were excised from the geranium plants, and while still fresh, the pedicels were removed at the top and bottom with sharp shears and separated. Pedicels were placed in a rigid plastic container (50-mL polypropylene centrifuge tube with screw cap), capped loosely, and frozen quickly in liquid nitrogen (−190°C). The tube was loosely capped to prevent shattering by the temperature differential, but the looseness also allowed some liquid nitrogen to flow into the tube. The tissue was processed immediately, or if needed, tubes containing pedicels were transferred to a −80°C freezer for long-term storage.

Trichome Preparation

At the time of isolation, tubes containing the intact pedicels were transferred to the liquid nitrogen tank to maintain them at cold temperatures and in close proximity to the laboratory bench for rapid preparation without thawing. The tube containing the intact pedicels was removed from the liquid nitrogen tank and approximately 2 to 3 cm^3 of finely powdered dry ice (prepared by wrapping a piece in clean paper towels and crushing with a hammer) was added to the tube. Immediately, the tube was loosely capped and vortexed (Vortex-Genie 2 mixer, model G-560, Scientific Industries, Bohemia, NY) at maximum speed, 3200 rpm, for approximately 1 min, which was sufficient time to shear all the trichomes from the pedicels. The trichomes at this point were either among the particles of dry ice or were stuck to the inside walls of the tube.

To recover the trichomes, the contents of the tube were poured onto a 25-cm diameter piece of fiberglass screen (1.4 mm/mesh) placed over a 600-mL beaker. The screen was momentarily shaken lightly over the beaker, letting the particles of dry ice and free trichomes fall into the bottom of the beaker, while retaining the trichome-free pedicles on the screen. The beaker contained whatever solvent or buffer was necessary for further tissue preparation. The trichome suspension in the beaker was immediately homogenized (Tekmar TR-10, 8-mm diameter probe). In addition, the tube used for vortexing retained trichomes adhering to the interior wall, and these were extracted by pouring the same solvent or buffer into the tube and homogenizing the contents with a wiping action of the Tekmar probe.

When the trichome-free pedicels were also to be extracted, they were taken from the screen before thawing, placed in a clean tube, and returned to the liquid nitrogen tank for temporary storage to maintain low temperature. Later, they were processed by grinding with liquid nitrogen in a mortar and pestle, added to the appropriate buffer, and homogenized. For lipid extractions, the maintenance of low temperature was not necessary, and the pedicels were simply homogenized in solvent.

The sequence of manipulations after removal of the unprocessed pedicels from liquid nitrogen until homogenization was conducted as rapidly as possible to prevent thawing of the cells which would have resulted in degradation. The addition of dry ice (−78°C) also helped maintain temperature.

RNA Isolation

Trichomes prepared in the above manner were used as a source of RNA. However, instead of placing the screen over a beaker, after vortexing, the trichomes were sieved directly into a mortar and pestle packed in dry ice. The trichomes were ground while still frozen and then thawed in a Tris buffer with proteinase K treatment (1). Subsequently, the extract was purified by phenol extractions, carbohydrate precipitation, and LiCl precipitation (1). The RNA was precipitated with ethanol and subsequently quantified by UV spectrophotometry (260 nm). The total RNA was passed through an oligo(dT) column to isolate mRNA (23). The mRNA was reverse transcribed with [^{32}P]dCTP to synthesize cDNA by a procedure scaled down from a hybridization probe (9). The cDNA was electrophoresed on an alkaline agarose gel and autoradiographed to monitor the length of cDNA strands synthesized.

The exact weight of the starting tissue was not determined because the thawing necessary for weighing allows RNase activity; however, in this experiment, 12 tubes of whole pedicels with trichomes were used for RNA isolation, which would yield an estimated 7.8 g fresh weight of trichome tissue.

Enzyme Activity

Proteins were extracted from trichomes isolated in the manner described above. Just after the vortexing step with powdered dry ice, the contents of the tube were poured out so that only the trichomes adhering to the interior wall of the tube remained. Immediately, a reducing Tris buffer (22) was poured into the tube, and the contents were homogenized. The extract was applied to filter paper wicks and inserted into 12% starch gels for electrophoresis (18).

The gels were run for approximately 5 h using two systems: citrate-morpholine buffer (pH 6.1, 25 mA, 120 V) and lithium-borate/Tris-citrate buffer (pH 8.3, 50 mV, 120 V) (18). The proteins were stained for activity in several common enzyme systems: esterases (nonspecific), glucose-6-phosphate isomerase, isocitrate dehydrogenase, phosphoglucomutase, and phosphogluconate dehydrogenase (28).

Lipid Isolation and Fatty Acid Analysis

Homozygous genotypes of zonal geraniums, previously characterized as arthropod-resistant (inbred 71–17–7) or susceptible (inbred 17–10–1) (31), were processed using the above technique. Instead of a plastic tube, a glass (scintillation) vial was used for the vortexing to remove trichomes because it was more resistant to the solvents used subsequently. The preparation vial with trichomes adhering to the inside surfaces was washed with 2 mL of chloroform:methanol (2:1, v/v) and vortexed for 2 min to extract the lipids from the trichome head cells. Then, 0.4 mL of methanol was added to the vial to change the specific gravity of the solution, causing the cellular debris to precipitate. After the vial was centrifuged, the supernatant was transferred to a clean 2-mL vial and dried under nitrogen with gentle heat.

The trichome-free pedicels were placed in a ground glass homogenizer with approximately 5 mL of chloroform:methanol and homogenized. Cellular debris was precipitated by adding 1.2 mL of methanol and centrifuging. The supernatant was removed and evaporated under nitrogen. In a modification of the Folch wash (8), the residue was dissolved in 2 mL of chloroform:methanol (2:1, v/v), and 0.4 mL water was added. The tube was vortexed for 1 min, and the bottom chloroform layer was removed to a 2-mL vial and evaporated under nitrogen with gentle heat. The subsequent lipid purification was identical for both the trichomes and the trichome-free pedicel preparations. The extracts of the trichomes were subsequently compared with the extracts of the pedicels from which those trichomes were removed.

The residue was redissolved in 0.5 mL of methanol, and the fatty acids were *trans*-esterified with the addition of 0.6 mL of 12.5% boron trifluoride in methanol. This standard derivatization reagent provides a fast, quantitative esterification of fatty acids (19) (BF_3/MeOH purchased from Supelco, Bellefonte, PA). The vials were heated 1 to 1.5 h in a 100°C oven. The esterified fatty acids were extracted by adding 0.5 mL of water, then 1.0 mL of hexane, and agitated. The upper hexane layer containing the methyl esters was placed in a 2-mL vial and evaporated under nitrogen.

The resulting methyl ester samples were redissolved in 100 μL of hexane and injected into a Hewlett-Packard 5890 gas chromatograph, operating in a splitless, off-column injection mode, and equipped with a 15 m RTX-2330 capillary column (Restek Corp., Bellefonte, PA; 0.25-mm i.d. column, 0.20-μm film thickness), with helium as the carrier gas at 7 p.s.i./20 mL/min, an injector temperature of 200°C, and a flame-ionization detector operating at 260°C. Sample peaks were recorded and integrated with a Shimadzu C5A integrator-recorder. Routine operating conditions utilized a 1-μL injection volume and a triple-ramped temperature program from 50 to 250°C (specifically: 50–150°C at 10°C/min, 150–190°C at 5°C/min, 190–250°C at 20°C/min, then held at 250°C for 10 min).

A standard fatty acid methyl ester mix (RM-3, Supelco) was used as a retention time standard. This mixture was supplemented with commercially available branched chain fatty acid methyl esters (Larodan, Uppsala, Sweden) and with ω5 fatty acid methyl esters which we prepared from the appropriate aldehydes.

The ω5 fatty acid methyl esters used as standards were prepared as follows. A *cis*-11 C16:1 aldehyde and a *cis*-13 C18:1 aldehyde (Sigma) were each separately oxidized by silver oxide (7, 27) to the free carboxylic fatty acid, extracted from the reaction mix with ethyl ether, washed with water, dried over anhydrous sodium sulfate, evaporated under nitrogen, and stored in hexane. A small aliquot was taken to verify on TLC (conditions as described in ref. 14) that the oxidation of the aldehyde to the acid was complete. The putative free fatty acids were methyl esterified by diazomethane in ethyl ether:methanol, and subsequently analyzed by GC (conditions as above). The free fatty acid products were purified from the oxidation mixture by preparative HPLC with a Supelcosil 5μm 4.6-mm LC8 DB column and solvent of acetonitrile:isopropanol:0.1% (w/v) phosphoric acid (500:220:300, v/v), pH 3.0, at 1-mL/min flow and wavelength detection at 212 nm. The eluting solvent from each fatty acid absorption peak was collected, reduced in volume under nitrogen gas, extracted with ethyl ether, washed with water, and dried over anhydrous copper sulfate. It was further repurified on HPLC with the same conditions except the phosphoric acid concentration in the mobile phase was increased to 325 parts. It was then recollected, reextracted, and stored in hexane. An aliquot of each free fatty acid was then methyl esterified by diazomethane in ethyl ether:methanol. The purity of the ω5 fatty acid methyl ester standards was analyzed by GC-MS for purity (conditions as described in ref. 14).

RESULTS AND DISCUSSION

Effectiveness of the Method

The stalk and head cells of tall glandular trichomes on geranium pedicels were completely removed by the technique. The trichomes as they appear adhering to the sides of the tube after the preparation are illustrated in Figure 1. No other contaminating plant tissues were present in the preparations. This photograph is typical in that the head cells usually separated from the stalk cells. An unprocessed pedicel with trichomes is shown in Figure 2, adjacent to a pedicel from which the trichomes were removed. After our method, the surfaces of the pedicels were completely free of trichomes. Similar microscopic examination allowed us to observe that the technique was also successful in isolating glandular trichomes of potato, tomato, squash, and velvetleaf.

Trichomes adhering to the walls of the tube constituted a preparation of exclusively stalk and head cells. More numerous were the trichomes that did not adhere but, instead, were dispersed among the particles of dry ice, forming a heterogenous preparation because there were also small fragments of other tissues. These results indicate that the most homogenous possible preparation can be obtained if only the trichomes adhering to the walls of the tube are used; if more tissue is needed, the trichomes scattered among the particles of dry ice can also be used.

The isolation of exclusively trichome head cells was improved by attention to a few details. More homogenous preparations were obtained when every effort was made to keep the tissue very cold and when the dry ice was as finely powdered as possible. Another detail was that, after the dry

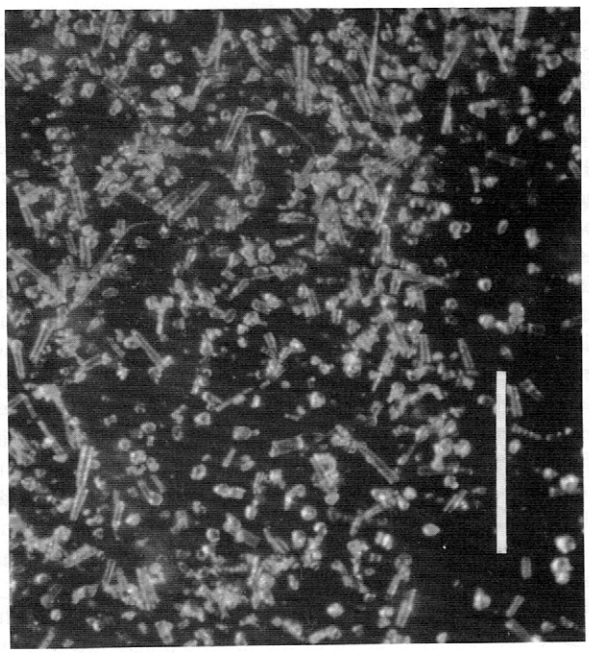

Figure 1. Stalk and head cells of geranium tall glandular trichomes as they appear after trichome preparation. Bar = 1 mm.

ice was powdered, it tended to condense water on its surface, making the particles larger and heavier. The water-coated dry ice was not as selective in removing only glandular trichome head cells. It tended to fracture other bits of tissue as well; therefore, only freshly powdered dry ice was used. Finally, adjusting the amount of vortexing time produced the desired type of preparation. Vortexing for a short time removed the most exposed tissue, resulting in a small amount of pure trichome head cells adhering to the walls of the tube. Vortexing for more time removed more tissue and resulted in more glandular head cells adhering to the walls of the tube; in addition, some stalk cells also adhered. Vortexing for even longer times fractured bits of the supporting tissue, but all the trichomes were certainly removed. Longer vortexing times were ideal when trichome-free preparations were desired. Optimal amounts of time for vortexing geraniums varied from 20 s to 3 min, depending on the firmness of the tissue from which the trichomes were being removed (leaf, sepal, or pedicel, respectively), and the quantity *versus* quality tradeoff desired for final use. The leaf or sepal was vortexed for shorter amounts of time to prevent it from shattering into small pieces that adhered to the walls of the tube along with the trichomes.

When leaves were used instead of sepals or pedicels, the procedure was modified slightly to accommodate the different shape. The fresh leaf was cut into 1- to 2-cm^2 pieces before freezing in liquid nitrogen to simplify its fit into the tube and to prevent tight folding which shielded trichomes from the dry ice.

The method was very effective in removing pubescences from plant surfaces. If specific tissues were chosen that contained several kinds of trichomes, then the preparation harvested those kinds of trichomes. For example, geranium leaves contain four types of trichomes (tall glandular, short glandular, tall spines, short spines), all of which are harvested by the method. Tissue that contained only one trichome type, *e.g.* geranium pedicels, yielded only that trichome type when processed with the method. The method was broadly applicable for harvesting trichomes from specific tissues that had the type(s) of trichome desired.

The method was somewhat selective for tissue that contained a mixture of trichome types, although the preparation was not as uniform. For example, on geranium leaves, 2 min of vortexing removed long spines but left short glandular trichomes, whereas 5 min vortexing removed most short glandular trichomes also. Similarly, for tomato leaves, the tetralobulate trichomes (type VI) were removed with short vortexing times, whereas the short spines were removed with longer vortexing times. Potato leaves contain many more spines than glandular trichomes, but still an enriched preparation of glandular trichomes was obtained by first vortexing for a short time to remove spines, quickly pouring the contents to a clean tube, vortexing again, and using the preparation of glandular trichomes adhering to the second vortexing tube.

A convenient way to empirically determine the optimal vortexing time for a specific situation was to try a few preparations with different time periods in a tube large enough to

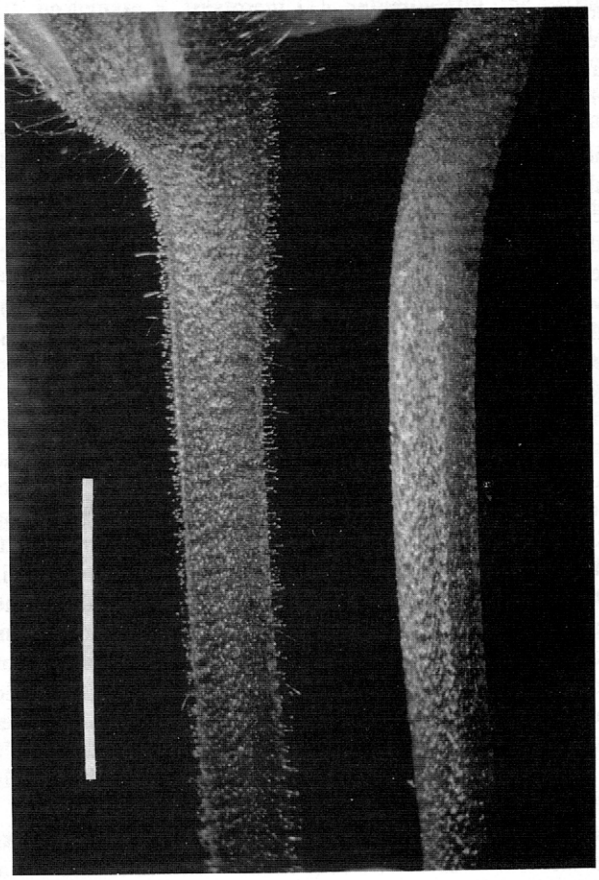

Figure 2. At left are the numerous glandular trichomes visible on a normal pedicel of a geranium floret, removed from an inflorescence. At right is a pedicel after the glandular trichomes have been removed. Bar = 1 cm.

hold a microscope slide. The preparation was done as usual, but a clean microscope slide was inserted into the tube just before vortexing; afterward, the slide was removed from the tube, thawed, and examined microscopically for the types of adhering cells. A 50-mL polypropylene centrifuge tube with screw cap conveniently held a standard 3- × 1-inch microscope slide.

Yield with the Method

The method will permit the isolation of a large quantity of tissue in a reasonable amount of time. A typical isolation procedure was as follows. A harvest of 125 fresh geranium inflorescences were dissected to remove the pedicels (tall glandular trichome-containing tissue) and placed in 50-mL tubes in liquid nitrogen. This yielded 11 tubes of tissue weighing 219.957 g fresh weight in 8 h. It required an additional 4 h to process the tissue through the isolation method in 11 batches. Tissues were separated into three fractions and subsequently dried at 50°C for 24 h for accurate quantification: pedicels, trichome-free (10.5714 g dry weight); trichomes, stuck to the walls of the tube (122.3 mg dry weight); and more trichomes, these sieved through the screen with the dry ice (228.8 mg dry weight). The total yield of trichomes was 7.15 g fresh weight, calculated from a water content of 95.1 ± 0.1% of whole pedicels with trichomes (the water content value obtained by subtracting combined dry from fresh weights). This procedure yielded 3.25 ± 0.10% pure trichome tissue from whole pedicels with trichomes. Of the trichome tissue harvested, 35.2 ± 1.7% of the trichomes were on the walls of the tube, and 64.8 ± 1.7% of trichomes were in the dry ice.

Integrity of the Trichome Tissue

Trichomes isolated by this method yielded 1.08 mg of total RNA and, after passage through an oligo(dT) column, 56 μg of mRNA. The purified mRNA was undegraded, as determined by autoradiography of an alkaline-agarose gel of cDNA from reverse transcription with a ^{32}P-labeled nucleotide. In this experiment, the technique yielded 0.014% total RNA from fresh trichome tissue.

Trichomes isolated by this technique can also be a source of active enzymes. Proteins extracted from the trichomes migrated during electrophoresis on starch gels and were visible after routine staining for a series of enzymes. Staining revealed that esterases (nonspecific), glucose-6-phosphate isomerase, isocitrate dehydrogenase, phosphoglucomutase, and phosphogluconate dehydrogenase were active in the trichome tissue.

Utility of the Method

The ability to isolate pure trichomes rapidly was useful for many applications, especially because the low temperature preserved the biochemical integrity of the cell contents. We demonstrate one application here, the determination of the fatty acid composition of glandular trichomes, trichome-free tissue, and intact leaf and pedicel tissue from geraniums previously characterized as spider mite-resistant or -susceptible plants (Table I). The most striking difference was the absence of ω5 fatty acids, either C16:1 or C18:1, in susceptible geraniums. This lack of ω5 fatty acids in susceptible geraniums is especially interesting because susceptible plants also lack appreciable quantities of ω5 anacardic acids (14), and ω5 fatty acids are biosynthetic precursors of ω5 anacardic acids (30).

Resistant geraniums are known to contain small amounts of ω5 fatty acids as well as high relative amounts of ω5 anacardic acids (30, 31). Our values for ω5 fatty acids in intact leaf and pedicel tissue ranged from 0 to only 2.3%, confirming the low concentrations reported in earlier studies (30). In contrast, high concentrations of ω5 fatty acid existed in the glandular trichome preparation. The unusual ω5 C16:1 fatty acid was present in trichomes isolated from resistant pedicels as 17.3% of total fatty acids. The ω5 C16:1 and C18:1 fatty acids together constituted 5.6 and 23.4% of total fatty acids

Table I. *Fatty Acid Composition of Resistant and Susceptible Geranium Tissues, with and without Trichomes*

Values are expressed as percentages of total fatty acids (mean ± SE). Other minor fatty acids detected in trace quantities are not presented here and account for small percentages for each tissue type. For indicating the position of the unsaturation, Δ11 and ω5 are equivalent alternatives in a C16:1 fatty acid. The minimum detectable amount is 0.1%.

Genotype	Source Tissue	Sample	Repetitions	C16:0	C16:1 Δ11 or ω5	C18:0	C18:1 Δ9 or ω9	C18:1 Δ13 or ω5	C18:2 ω6,9	C18:3 ω3,6,9
Resistant	Leaf	Intact	2	22.7 ± 0.92	0.5 ± 0.04	1.4 ± 0.04	3.0 ± 0.48	0.0 ± 0.02	19.1 ± 0.81	46.7 ± 0.33
	Leaf	No trichomes	2	22.8 ± 0.08	0.0 ± 0	1.6 ± 0.11	2.6 ± 0.43	0.0 ± 0	19.8 ± 0.04	47.4 ± 0.43
	Leaf	Trichomes	2	19.0 ± 2.65	2.5 ± 0	10.2 ± 1.23	4.2 ± 0.20	3.1 ± 0.21	25.2 ± 2.27	20.6 ± 1.87
	Pedicel	Intact	2	28.1 ± 0.62	2.3 ± 0.05	2.4 ± 0.19	3.8 ± 0.22	0.6 ± 0.01	36.5 ± 0.29	24.3 ± 0.36
	Pedicel	No trichomes	8	27.9 ± 2.62	0.0 ± 0	3.1 ± 0.28	4.2 ± 0.51	0.0 ± 0	39.7 ± 0.96	22.9 ± 0.98
	Pedicel	Trichomes	10	22.3 ± 2.91	17.3 ± 1.95	8.4 ± 1.81	5.3 ± 0.40	6.1 ± 0.86	23.9 ± 2.50	7.2 ± 0.86
Susceptible	Leaf	Intact	3	25.6 ± 1.48	0.0 ± 0	2.3 ± 0.38	5.5 ± 1.40	0.0 ± 0	19.1 ± 1.19	41.4 ± 2.52
	Leaf	No trichomes	2	21.4 ± 1.17	0.0 ± 0	1.7 ± 0.21	3.8 ± 0.10	0.0 ± 0	19.3 ± 0.06	48.7 ± 0.55
	Leaf	Trichomes	1	21.7	0.0	18.7	8.3	0.0	28.2	15.0
	Pedicel	Intact	1	31.8	0.0	1.6	3.8	0.0	35.3	25.6
	Pedicel	No trichomes	4	29.9 ± 3.20	0.0 ± 0	2.6 ± 0.63	3.5 ± 0.25	0.0 ± 0	32.8 ± 0.90	29.4 ± 3.06
	Pedicel	Trichomes	7	21.5 ± 1.44	0.0 ± 0	7.1 ± 2.14	7.3 ± 1.33	0.0 ± 0	33.3 ± 1.91	12.3 ± 1.13

in glandular trichome preparations of leaves and pedicels, respectively.

Neither the C16:1 nor the C18:1 ω5 fatty acids were present in the trichome-free pedicels or trichome-free leaves (Table I). Both ω5 fatty acids were present exclusively in the trichomes. The intact pedicel with trichomes contained the ω5 C18:1 fatty acid as 0.6% of total fatty acids, an order of magnitude smaller than 6.1% found in pure trichomes. These results demonstrate that comparing the amount in intact pedicels, 0.6%, and trichome-free pedicels, 0%, gave no indication of the concentration present in the trichome, 6.1%. The same comparisons were true for the ω5 C16:1 fatty acid. These data indicate that the ω5 fatty acid concentration in trichomes could not simply be deduced by subtracting the concentrations in intact *versus* trichome-free tissue. Thus, methods that do not actually purify trichome tissue to directly measure its unique physiology would result in totally misleading conclusions for ω5 fatty acids. Our method allows direct access to trichome tissue, making subtractive comparisons unnecessary.

Some interesting trends were evident for other fatty acids present in the trichomes (Table I). Linolenic acid, C18:3 ω3, 6, 9 (or Δ9, 12, 15), occurred at less than half the concentration in trichomes than in trichome-free tissue or in intact tissue. For example, intact leaves of susceptible geraniums contained 41.4% of total fatty acids as linolenic acid, trichome-free leaves contained 48.7%, and trichomes contained only 15.0%. Relative concentrations of palmitic acid, C16:0, followed the same trend. Conversely, stearic acid, C18:0, and oleic acid, C18:1 ω9 (or Δ9), were found in higher concentrations in the trichomes than in the supporting leaf or pedicel tissue. Pure trichomes isolated from the leaves of susceptible geraniums contained 18.7% of total fatty acids as stearic acid, whereas trichome-free leaves contained 1.7%, and intact leaves contained only 2.3%. Linoleic acid, C18:2 ω 6, 9 (or Δ9, 12), occurred in considerably higher concentrations in pedicels than in leaves of both resistant and susceptible geraniums. In contrast, there was more linolenic acid in the leaves than in the pedicels.

CONCLUSION

The differing concentrations of individual fatty acids in the trichomes *versus* the trichome-free leaves or trichome-free pedicels indicate that the trichome head cell has a unique biochemistry distinct from leaf or pedicel tissue. The presence of ω5 fatty acids exclusively in the trichomes and not in the trichome-free supporting tissue indicates that their most likely site of biosynthesis is in the trichome. This knowledge allows us to pursue the trichome itself for the specialized enzyme system producing the unusual ω5 desaturation in the fatty acid. Furthermore, the finding of an abundance of ω5 fatty acids exclusively in trichomes of resistant geraniums supports other evidence that ω5 fatty acids are precursors of ω5 anacardic acids (30). Because ω5 fatty acids occur only in trichomes, and ω5 anacardic acids make up most of the trichome exudate, it is likely that the biosynthesis of the anacardic acid from the fatty acid occurs in the trichome. Thus, we also can investigate the trichome for the site of the enzyme system responsible for anacardic acid biosynthesis.

Being able to separate the trichomes from the trichome-free pedicels of geraniums allows us to make definitive measurements of location and abundance, and postulate sites of biochemical reactions. This method has been extremely useful in our investigations of the location of the site of anacardic acid biosynthesis which imparts resistance or susceptibility to spider mites in geraniums. We believe that this reported isolation has broad applicability for many types of biochemical or molecular investigations of glandular trichomes. Because the method allowed us to isolate pure glandular trichomes from every plant attempted, we believe it will be generally applicable for many taxa.

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